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Filopodia in cell adhesion, 3D migration and cancer cell invasion

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This review discusses recent advances in our understanding of the role filopodia and filopodia-like structures in cell adhesion and three dimensional (3D) cell migration both *in vitro* and *in vivo*. In particular, we focus on recent advances demonstrating that filopodia are involved in substrate tethering and environment sensing *in vivo*. We further discuss the emerging role of filopodia and filopodial proteins in tumor dissemination as mounting *in vitro*, *in vivo* and clinical evidence suggest that filopodia drive cancer cell invasion and highlight filopodia proteins as attractive therapeutic targets. Finally, we outline outstanding questions that remain to be addressed to elucidate the role of filopodia during 3D cell migration.

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Introduction

The ability of cells to migrate *in vivo* is necessary for many physiological processes including embryonic development, tissue homeostasis and wound healing. Cell migration is also implicated in distinct pathological conditions such as inflammation and cancer metastasis. The ability of cancer cells to disseminate from the primary site and form distant metastases is the main cause for cancer-related morbidity in patients with solid tumors. Therefore, much effort has been invested in the identification of the signaling pathways and the cellular structures involved in the migratory process of cells within a 3D environment. Consequently, several mechanisms driving collective cell movement in addition to distinct modes of single cell migration (amoeboid, mesenchymal, lobopodial and pseudopodial) have been described [1,2•].

Regardless of the migratory mode employed, effective cell motility requires cells to adapt to, interact with, and often modify their surrounding extracellular matrix (ECM). Trans-membrane receptors such as integrins and growth factor receptors cluster and recruit cytoplasmic adapters and signaling molecules (i.e. small GTPases, kinases) to assemble adhesion complexes at sites of cell–ECM interaction. These adhesion complexes act as anchorage sites and enable the coordinated regulation of plasma membrane dynamics, by providing a physical link to, and through the active remodeling of, the actin cytoskeleton. In addition, mechanical forces exerted at these anchorage points as well as membrane-bound and secreted proteases allow cells to restructure their microenvironment. Most of these processes will be discussed in detail in other articles in this issue. In this review, we will focus on the emerging role of filopodia in 3D cell migration, as multiple studies, from different biological systems, report that cell motility through complex 3D microenvironments requires the efficient probing of the cell surroundings (ECM, neighboring cells, cytokines) via these specialized actin-rich protrusions.

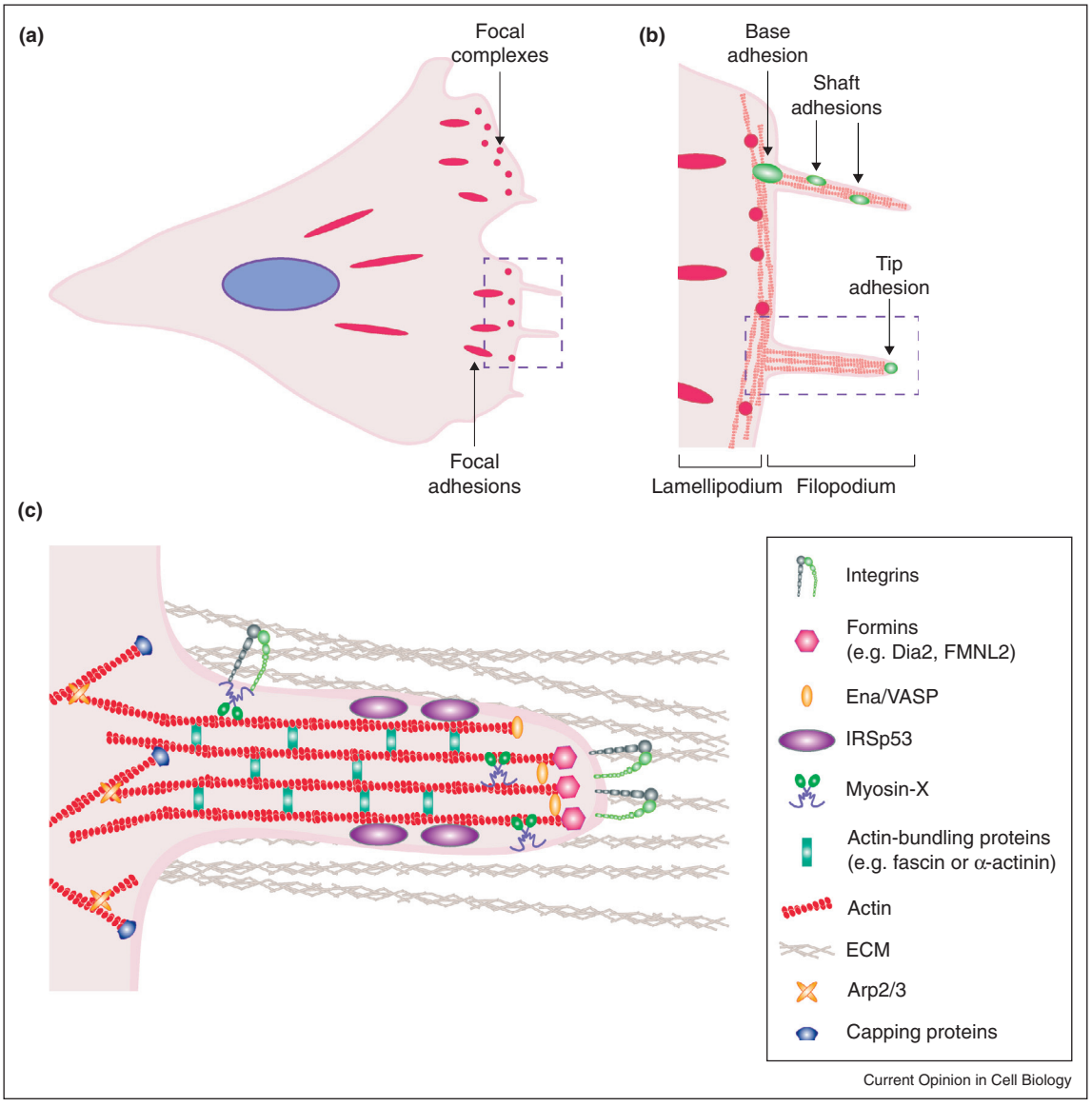
Filopodia, cell adhesion and environment sensing

Filopodia are thin, finger-like and highly dynamic actin-rich membrane protrusions that extend out from the cell edge. Extension of a filopodium is driven by linear polymerization of actin filaments mediated by proteins such as formins and regulated by various small GTPases of the Rho family (i.e. Rac1 and Cdc42), actin capping proteins, actin regulators (Ena/Vasp), and I-Bar proteins (IRSp53) (see [3–5] for review). Within a filopodium, actin-bundling proteins, such as fascin, villin or α-actinin [6], tightly pack parallel actin filaments into bundles with their barbed ends facing toward the plasma membrane. This unidirectional organization allows molecular motors, such as myosin-X (MYO10), to transport various proteins, including transmembrane receptors, along actin filaments to the tips of filopodia to participate in filopodia maintenance and stabilization, environment and cue sensing, establishment of cell–cell junctions and long-distance transport (Figure 1). The transport of receptors to filopodia tips is critical in many physiological processes including early embryonic development [7], tissue patterning [8,9], dendritic spine formation and stabilization [4], and mounting of an effective immune response [10].

In the context of cell migration, the transport of cell–cell and cell–ECM adhesion receptors as well as cytokine receptors to the tips of filopodia allows the cell to efficiently probe the surrounding environment. The transport of

cell–cell adhesion receptors to filopodia tips has been implicated in the formation and reinforcement of cell–cell junctions [11,12] and therefore could contribute to collective cell migration or to contact inhibition of locomotion during single cell migration [13••]. However, the significance of filopodia-mediated intercellular communication during cell motility has yet to be elucidated. A more defined role for filopodia in cell migration has been linked

Figure 1



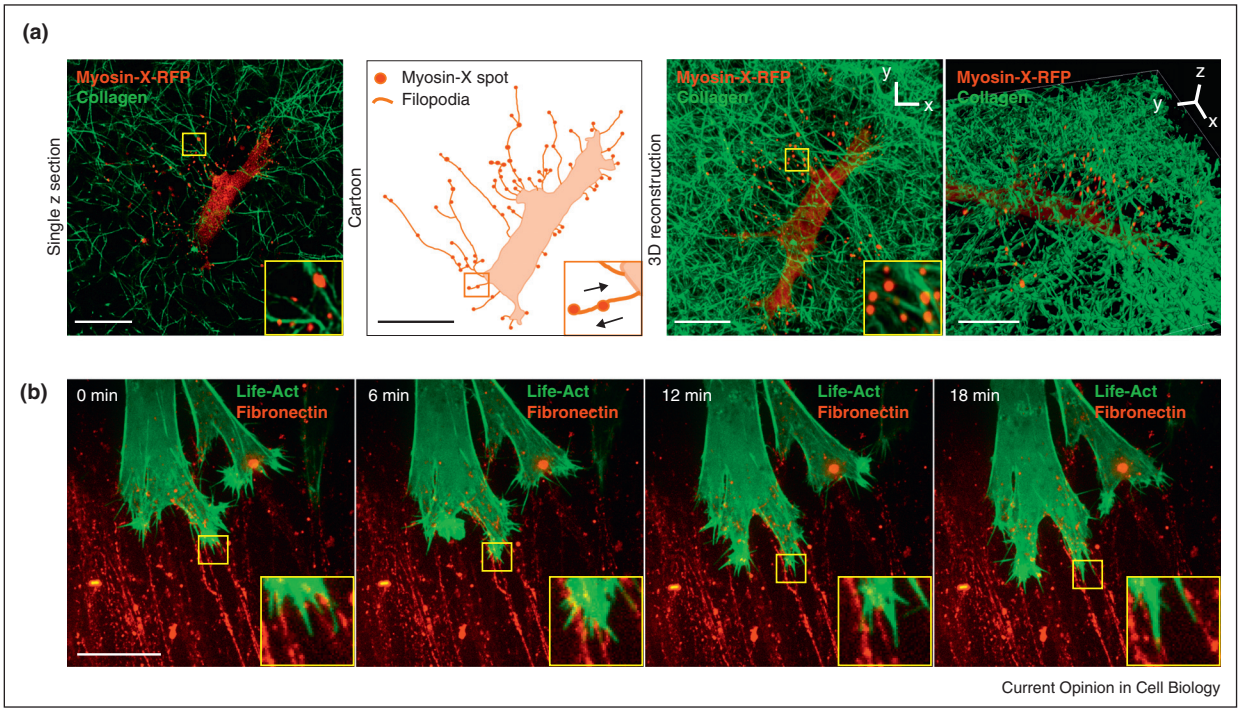
Schematic representation of filopodia during cell migration on a planar substrate. **(a)** During cell migration, receptor-mediated cell–ECM interactions induce the assembly of dynamic and heterogeneous cytoplasmic adhesion platforms, termed adhesion complexes. As cells migrate in 2D, different types of adhesion complexes (nascent adhesions, focal adhesions and fibrillar adhesions) can be discerned based on maturation stage, sub-cellular distribution and protein composition. These support cell–ECM adhesion and function as signaling platforms that tightly control cell behavior. In particular, adhesion-mediated signaling at the leading edge regulates Arp2/3-mediated actin remodeling and polymerization to drive lamellipodia membrane protrusions and forward cellular movement. In addition, linear actin polymerization leads to the formation of filopodia that explore the cell surroundings. **(b)** Filopodia probe the ECM by assembling specialized adhesion complexes at specific sub-filopodial locations, namely 'tip adhesions', 'shaft adhesions' and 'base adhesions'. **(c)** Filopodia formation is facilitated by proteins such as the insulin-receptor substrate p53 (IRSp53) (or other inverse (I)-BAR domain-containing proteins) which deform and/or tubulate the plasma membrane, and by the motor activity of myosin-X which triggers actin fiber convergence at the cell periphery. Within a filopodium, actin crosslinking protein such as fascin or α-actinin bundle actin filaments together and formins including Dia2 (diaphanous-related formin-2) and/or actin regulators such as Ena/VASP (enabled/vasodilator-stimulated phosphoprotein) promote actin filament elongation. Myosin-X also transports various proteins to filopodia tips, including adhesion receptors such as integrins [22] or VE-cadherin, with key roles in filopodia maintenance, environment sensing, establishment of cell–cell junctions and long-distance transport.

to ECM tethering and probing through the transport of cell–ECM adhesion receptors to filopodia tips. This role of filopodia in ECM sensing will be a key focus of this review.

By transporting integrins, a key family of adhesion receptors, filopodia play a central role in modulating cell adhesion, and unsurprisingly many cell types use filopodia during the early phase of spreading [14•,15–17]. Classically, in two-dimensional (2D) environments, integrin-mediated adhesion complexes are assembled in the ruffling lamellipodium (nascent adhesions) and, upon mechanical stress, mature into larger structures (focal adhesions) (Figure 1) (reviewed in [18••]). In cells migrating in 2D, additional substrate anchorage sites have been observed at the base, the shaft and/or the tip of individual filopodia that project out from the advancing lamellipodium [19] (Figure 1) [20,21]. However, the role and/or molecular composition of these filopodia-specific adhesion complexes remains poorly defined. Adhesion sites formed at the shaft and at the tip are integrin-mediated [15–17] and are thought to be critical in promoting filopodium stability and limiting filopodia growth [20]. The size and shape of the adhesions located at the base of filopodia closely resemble those of focal adhesions found in lamellipodia and are likely to have similar composition and function. Importantly, recent evidence demonstrated that filopodia shaft adhesions can mature into focal adhesions upon lamellipodia advancement [24] suggesting that the adhesions found at the shaft and/or tip of filopodia could be representative of nascent adhesions.

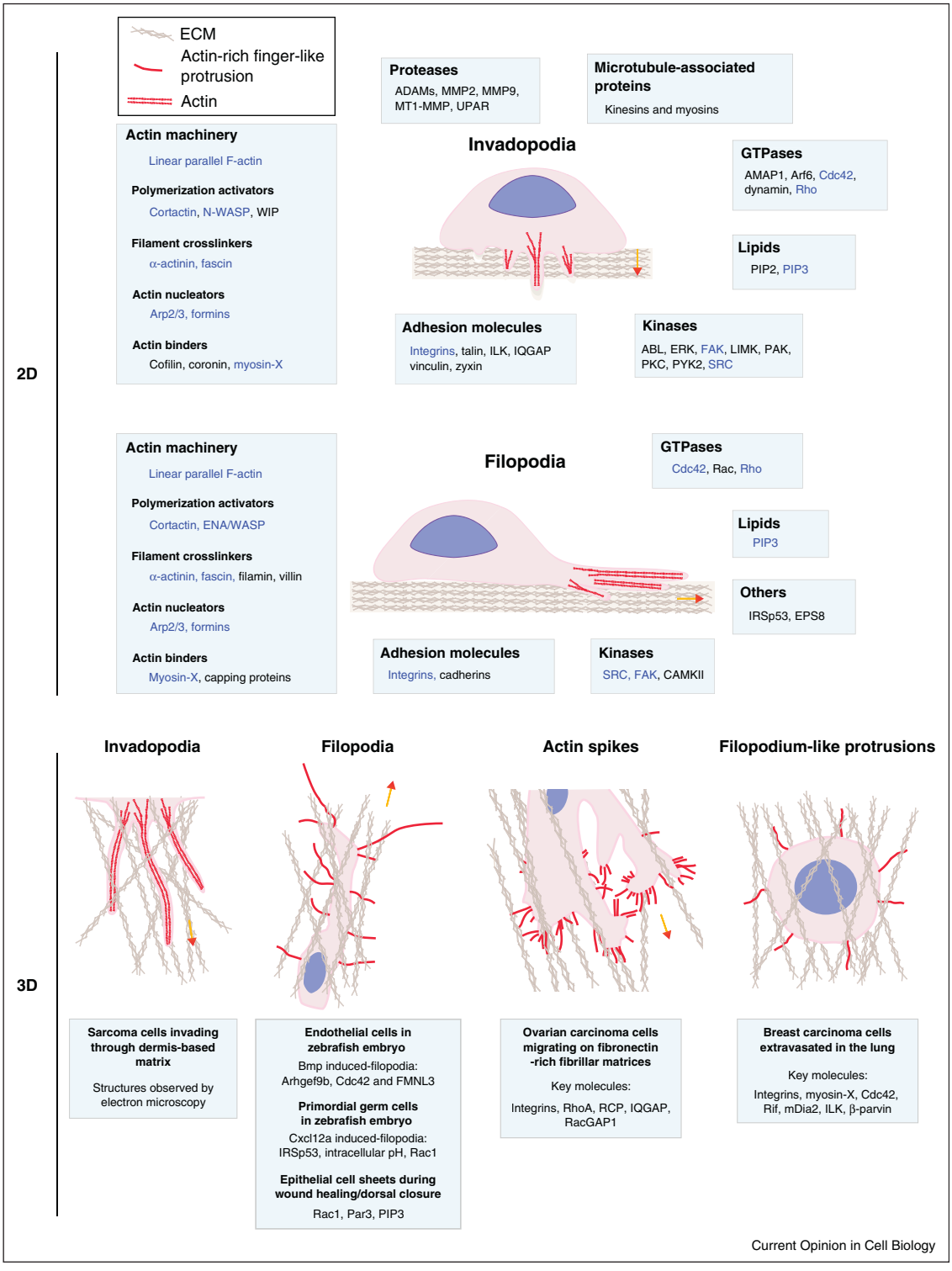
Filopodia have been described to play a key role in substrate tethering and environment sensing. In 2D, in fibroblasts, filopodia appear to be required for haptotaxis and to be dispensable for chemotaxis [19] suggesting that one of the key functions of filopodia is to probe the ECM. In particular, cells may employ filopodia to recognize ECM topography (Figure 2) [15] as well as ECM stiffness [25,26]. However, how filopodia probe the matrix environment and how this information is relayed during cell migration remains poorly defined. In this context, filopodial tip adhesions, as the first point of contact with new ECM, are likely to make a critical contribution. Indeed, adhesion complexes not only provide anchorage but also represent integrin heterodimer-ligand-specific and/or ECM-ligand-specific signaling nodes [27,28] and mechanosensing stations [29,30], and, therefore constitute ideal signaling platforms for ECM recognition. The maturation of filopodia shaft adhesions into focal adhesions [24] could further explain the importance of filopodia during

Figure 2



Cancer cells assemble filopodia as they migrate in 3D. **(a)** A confocal image of an MDA-MB-231 breast cancer cell invading through a 3D fibrillar collagen gel (green: collagen; red: myosin-X-RFP for visualization of the filopodia tips). A single Z-section, an associated cartoon representation, and Imaris-generated 3D reconstruction images are displayed (scale bars = 20 μm). In the cartoon, the arrows in the magnified area represent the movement of myosin-X spots and their cargo along the filopodia shaft. **(b)** Live-cell imaging of an ovarian carcinoma cell (A2780) migrating invasively on fibronectin-rich fibrillar matrices (red: fibronectin; green: Life-Act GFP for visualization of the actin cytoskeleton). Images were acquired using a spinning-disk microscope and Z-projections are displayed (scale bars = 20 μm).

Figure 3



Filopodia, invadopodia and filopodia-like structures. In 2D, cells form well-defined finger-like, actin-rich structures including filopodia and invadopodia. Filopodia are transient and extend out from the advancing lamellipodium, whereas invadopodia are more stable, localize beneath the cell body and possess substrate degradation properties. Filopodia are classically associated with proteins such as myosin-X and fascin, while invadopodia contain proteins such as cortactin and the ECM-degrading protease MT1-MMP (see [67] for review). Some other key components of filopodia or invadopodia are displayed (list non-exhaustive) and organized by functional categories (inspired/adapted from [67]). Despite their differences in sub-cellular localization and substrate degradation, the molecular machinery responsible for the formation of filopodia or

haptotaxis [19]. In excitable cells such as neurons, ion channel signaling is critical for filopodia tethering (reviewed in [31]). However, the contribution of ion channels to filopodia function during cell migration remains to be determined.

In addition to the activation of signaling pathways, filopodia tethering also triggers a mechanical response by producing retraction forces [19,16,27,28]. In studies using optical tweezers and ECM-coated beads or measuring the deflection of nanowires, individual filopodium were shown to generate traction forces in an order of 5–25 pN with a maximum potential of up to 2 nN [19,16,27,28] (reviewed in [34]). In comparison, actomyosin-mediated contractility exerted at focal adhesions in 2D transmits stronger forces that are in the nano-Newton range [35]. Filopodial traction forces are thought to be generated by a combination of actin retrograde flow and helical bundling of actin filaments within the filopodium shaft [32•,33]. However, the role of filopodial retraction forces in ECM sensing and deformation, especially during 3D cell motility, requires further investigation.

Filopodia during cell migration *in vivo*

Filopodia and filopodial proteins have been principally studied in 2D, however, cells migrating on planar substrates often display only sparse and transient filopodia that are rapidly taken over by the advancing lamellipodium [15]. In contrast, extensive use of filopodia or actin-rich filopodia-like structures has been documented in several cell types as they migrate in 3D or *in vivo* (Figures 2 and 3).

Perhaps the best studied example of filopodia-like structures *in vivo* is associated with the process of endothelial sprouting and angiogenesis. Soluble growth factors such as vascular endothelial growth factor (VEGF) or bone morphogenetic proteins (Bmp) induce filopodial protrusions in the endothelial tip cell leading the sprout invasion in the surrounding ECM [36•,37]. The molecular machinery responsible for the formation of VEGF-induced filopodia is not fully understood and is mostly supported by experiments performed in 2D. However, small GTPases of the Rho family, actin nucleators and regulators as well as glycolytic enzymes regulating the actin cytoskeleton at the cell periphery [38] have been implicated in the process (see [39••] for review). The mechanism by which Bmp promotes filopodia formation was elucidated *in vivo* and was shown to involve Cdc42 guanine nucleotide exchange factor 9b (Arhgef9b), Cdc42 and formin-like 3 (FMNL3) [37]. The previously ascribed role of filopodia as fundamental regulators of tip cell guidance during angiogenesis [3,4], has now been challenged by several recent studies [40,41]. In zebrafish embryo, inhibition of filopodia formation appears not to alter endothelial tip cell directionality but rather to impede migration speed [40]. In addition, Bmp, which promotes filopodia formation, does not function as a guidance cue but instead increases endothelial cell motility [37]. Together these data suggest that, during angiogenesis, filopodia may not be required for chemotaxis but rather support migration, possibly by probing the ECM.

In zebrafish embryo, primordial germ cells (PGCs) assemble filopodia as they migrate toward the chemokine Cxcl12a [42]. Cxcl12a cues promote cellular polarization by activating the filopodial initiator IRSp53, resulting in an increased number of transient filopodia toward the Cxcl12a gradient. These transient filopodia amplify Cxcl12a-mediated signaling which leads to a localized increase in cellular pH and activation of the small GTPase Rac1. These filopodia appear to be critical for Cxcl12a-guided migration as inhibition of their formation, by interfering with IRSp53 function, results in the loss of PGC directional migration [42].

Filopodia have also been suggested to be functionally important at the edges of migrating epithelial cell sheets during wound healing and dorsal closure [43,44]. In these contexts, filopodia are not only critical for the efficient bonding of the two colliding epithelial sheets but also for appropriate tissue alignment and patterning [43]. This process was shown to require the small GTPase Rac1 [45], the cell polarity regulator Par3 and phosphatidylinositol (3,4,5)-trisphosphate (PIP3) [46]. Filopodia have also been observed, *in vivo*, in migrating neuronal crest cells [47] and blood cells such as macrophages [48,49••].

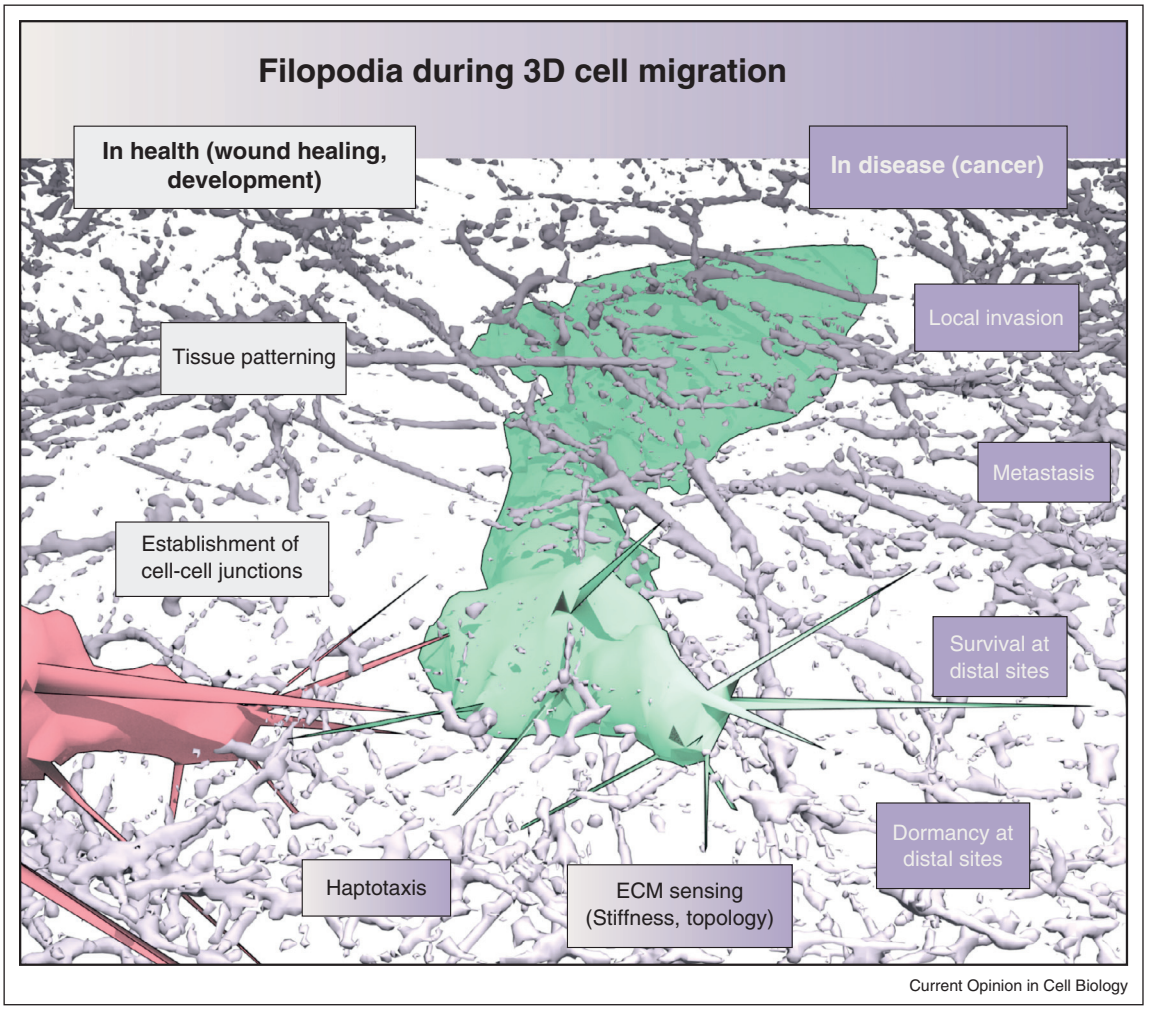
(Figure 3 Legend Continued) invadopodia share many similarities (some common molecules are highlighted in blue; list non-exhaustive). For instance, the invadopodial protein cortactin is involved in filopodia formation [68] and the filopodial proteins myosin-X and fascin also contribute to invadopodia formation [54,56]. In 3D and *in vivo*, cells form filopodia-like protrusions, however it is currently unclear whether these structures are comparable to the filopodia or invadopodia described in 2D. In addition, it is possible that, due to a lack of clear classification criteria, similar structures have acquired different names. The nomenclature used to describe filopodia-like protrusions in 3D includes filopodia [37,40,42], invadopodia [69], filopodium-like protrusions [50,51] and actin spikes [2]. The molecular machinery associated with these filopodia-like protrusions is poorly understood but the known components are displayed. ADAM, a disintegrin and metalloprotease; AMAP1, a multiple-domain Arf-GAP Protein 1; ARF6, ADP-ribosylation factor 6; CAMKII, Calcium/calmodulin-dependent protein kinase II; Cdc42, cell division control protein 42 homolog; ERK, extracellular signal-regulated kinase; FAK, focal adhesion kinase; IQGAP, IQ motif containing GTPase activating protein; LIMK, LIM-domain kinase; MMP, matrix metalloprotease; MT1MMP, membrane type 1 MMP; N-WASP, neural Wiskott–Aldrich syndrome protein; PAK, p21-activated kinase; PKC, protein kinase C; PIP2, phosphatidylinositol-3,4-bisphosphate; PIP3, phosphatidylinositol-3,4,5-triphosphate; RacGAP1, Rac GTPase activating protein 1, RCP, Rab-coupling protein; UPAR, urokinase plasminogen activator surface receptor; WIP, WASP-interacting protein.

Filopodia and cancer cell invasion

As filopodia are widely used by migrating cells *in vivo*, it is perhaps not surprising that filopodia or actin-rich filopodia-like structures have been observed in cancer cells, regardless of their mode of migration, as they invade through 3D micro-environments [1] (Figures 2 and 3). For instance, in ovarian cancer cells, an increased number of filopodia-like structures (termed actin spikes), induced by activation of the small GTPase RhoA, correlate with invasiveness [2]. In addition, in mice, following tail-vein injection, cancer cells that have extravasated into the lung display filopodia-like protrusions (termed filopodium-like protrusions). These filopodium-like protrusions contain β1 integrin along their shafts and their formation is regulated by myosin-X, the small GTPases of the Rho family Cdc42 and Rif, the formin mDia2 and by the focal adhesion regulators integrin-linked kinase (ILK) and β-parvin (Figure 3) [50,51]. These filopodia-like protrusions appear to be critical not only for driving cancer cell metastasis, but also for promoting survival and proliferation of the disseminated carcinoma cells at a secondary organ [50].

Clinical data also suggest a role for filopodia during cancer cell dissemination as several filopodial proteins are upregulated in human cancer and are required for cancer cell invasion *in vitro*. The best studied example is fascin, an actin-bundling protein that promotes filopodia assembly. Fascin is not expressed in healthy epithelium [6] but is upregulated in several aggressive and metastatic cancers of epithelial origin. Additionally, fascin levels have been shown to be high especially at the invading edges of

Figure 4



Filopodia during 3D cell migration. A cartoon representation of cancer cells invading through 3D fibrillar collagen. The cell shape and the collagen network were exported directly from confocal images using ImageJ and rendered using Blender. Filopodia-like structures are exaggerated to highlight the importance of these actin-rich protrusions during 3D cell migration in health and disease. The most important and established biological functions of filopodia in 3D are highlighted: gray boxes indicate roles in normal physiology and purple boxes indicate roles in pathological conditions such as cancer.

tumors and increased fascin levels often correlate with poor survival [52]. Fascin over-expression leads to increased migration and invasion both in 3D and in animal models [53], whereas fascin gene silencing leads to reduced invasiveness [54]. In addition, loss of fascin gene in a mouse model of metastatic pancreatic ductal adenocarcinoma (PDAC) promotes survival and lowers tumor burden [55••]. In line with these data, fascin has been detected in human PDAC samples with high fascin levels correlating with poor clinical outcome. Additionally, induction of fascin expression has been demonstrated during epithelial-mesenchymal transition downstream of slug [55••]. Therefore, it is likely that fascin, when expressed, participates in the early phase of cancer cell dissemination.

Myosin-X, another critical regulator of filopodia function, is up-regulated in breast cancer and correlates with poor prognosis [14•,56]. In cells, myosin-X over-expression promotes filopodia formation [57] and leads to increased cell invasion [14•], whereas myosin-X gene silencing inhibits cancer cell invasion both in 3D and in animal models [14•,56]. Myosin-X is thought to drive cell invasion by transporting integrin receptors to the tips of filopodia to tether the ECM [14•,23•]. Myosin-X gene expression is induced by gain-of-function mutations of P53 [14•], a common feature of metastatic human cancers [58], suggesting that myosin-X could drive cancer cell metastasis in multiple cancer types. Other filopodial proteins that have been implicated in human cancer and/or in the regulation of cell invasion include the filopodial initiators IRSp53 and EPS8 [17,59], the formins FMNL2 and mDia2 [60–62], the cytoskeletal regulators Ena/VASP [63] and the long isoform of CRMP-1 [64•].

Conclusion and perspectives

Here, we have reviewed some recent advances describing the contribution made by filopodia to environment sensing, 3D cell migration and cancer cell invasion. There is an increasing body of evidence demonstrating that cells use filopodia or filopodia-like protrusions, in 3D or *in vivo*, for ECM and environment sensing (Figure 4). However, our knowledge of filopodia structure, composition and dynamics is based principally on experiments performed in 2D and it is currently unclear how these findings translate as cells migrate in 3D. Thus, several important areas of investigation remain. One priority will be to better characterize the filopodia-like protrusions observed in 3D and to compare these to the filopodia and the structurally related matrix degrading invadopodia that are observed in 2D (Figure 3). In addition, it will be important to determine how filopodia probe complex 3D ECM structures and to identify the chemical and/or mechanical signals transmitting the information back to the cell to direct cell migration. These studies will be greatly facilitated by the development of new technologies that allow the efficient imaging of actin structures *in vivo* [65•] and by the development of software that identify and track filopodia dynamics [66•].

As cells migrate, filopodia are likely to not only probe and/or modify the surrounding ECM but also to interact with other cells and therefore to facilitate intercellular communication. However, the contribution of filopodia-mediated intercellular communication to forward movement has yet to be studied. Therefore, other important areas of investigation would be to assess the potential of filopodia to promote collective cell migration, contact inhibition of locomotion or tumor–stroma interactions.

Finally, filopodia and filopodial proteins make a critical contribution to cancer metastasis and therefore constitute attractive therapeutic targets to block cancer dissemination. Thus, an important focus for the future will be to identify compounds that inhibit filopodia formation and to assess their efficiency to block metastases both *in vitro* and *in vivo*.

Acknowledgements

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